AMENDMENTS TO THE SPECIFICATION

On page 2, line 6, please replace the text with the following paragraph:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Application Ser. No. 09/005,985, filed on January 12, 1998, now US Patent 6,168,948, which application claims the benefit of U.S. Provisional Application No. 60/043,490, filed Apr. 10, 1997. This application is a continuation-in-part of U.S. application Ser. No. 08/992,025, filed Dec. 17, 1997, now abandoned; and is a continuation-in-part of U.S. application Ser. No. 08/589,027, filed Jan. 19, 1996, now U.S. Pat. No. 5,856,174; and is a continuation-in-part of U.S. application Ser. No. 08/671,928, filed Jun. 27, 1996, now U.S. Pat. No. 5,922,591, which claims the benefit of U.S. Provisional Application No. 60/000,703, filed Jun. 29, 1995, and U.S. Provisional Application No. 60/000,859, filed Jul. 5, 1995. Each of these applications is incorporated herein by reference in its entirety for all purposes.

On page 5, line 17 through page 9, line 22, please replace the text with the following:

Figs. 8A and 8B are schematic illustrations ~~Fig. 8 is a schematic illustration~~ of a side and top view of a base-unit for use with a miniature integrated device.

Fig. 9A ~~Fig. 10A~~ is a gel showing a time course of an RNA fragmentation reaction. Fig. 9B ~~Fig. 10B~~ is a gel showing a comparison of the product of an in vitro transcription reaction in a microchamber vs. a control (test tube). Fig. 9C ~~Fig. 10C~~ is a comparison of the PCR product produced in a PCR thermal cycler and that produced by a microreactor.

Fig. 10 ~~Fig. 11~~ shows an embodiment of a reaction chamber employing an electronic pH control system.

Figs. 11A-C ~~Figs. 12A-C~~ show a schematic representation of a miniature integrated device employing a pneumatic fluid direction system utilizing a gas permeable fluid barrier bound vents, e.g., a poorly wetting or hydrophobic membrane, and pneumatically controlled valves. Fig. 11A ~~Fig. 12A~~ shows an embodiment of a single chamber employing this system. Fig. 11B ~~Fig. 12B~~ is a schematic illustration of a debubbling chamber for linking discrete fluid plugs that are separated by a gas bubble. Fig. 11C ~~Fig. 12C~~ schematically illustrates this system

in an integrated device having numerous chambers, including degassing chamber, dosing or volumetric chamber, storage and reaction chambers. Fig. 11D ~~Fig. 12D~~ is an illustration of an injection molded substrate which embodies the system schematically illustrated in Fig. 11C ~~Fig. 12C~~.

Fig. 12 ~~Fig. 13~~ is a schematic representation of a device configuration for carrying generic sample preparation reactions.

Fig. 13 ~~Fig. 14~~ is a schematic representation of a device configuration for carrying out multiple parallel reactions.

Fig. 14 ~~Fig. 15~~ shows a demonstration of integrated reactions in a microfabricated polycarbonate device. Fig. 14A ~~Fig. 15A~~ shows the layout of the device including the thermal configuration of the device. Fig. 14B ~~Fig. 15B~~ shows the results of PCR amplification and subsequent in vitro transcription within the chambers of the device.

Fig. 15 ~~Fig. 16~~ schematically illustrates a deformable high capacity nucleic acid extraction device incorporating a porous material for extracting nucleic acids from samples.

Fig. 16 ~~Fig. 17~~ is a side sectional view of a miniaturized reactor device incorporating a positive displacement fluid movement scheme.

Fig. 17A ~~Fig. 18A~~ is a top plan view of the pneumatic portion of the reactor device of Fig. 16 ~~Fig. 17~~.

Fig. 17B ~~Fig. 18B~~ is a top plan view of the fluid portion of the reactor device of Fig. 16 ~~Fig. 17~~.

Fig. 18 ~~Fig. 19~~ schematically illustrates an affinity based nucleic acid extraction device incorporating a textured wall.

Fig. 19 ~~Fig. 20~~ illustrates an allele-specific purification device according to the present invention.

Fig. 20 ~~Fig. 21~~ is a schematic representation of a miniaturized device for performing rapid thermal cycling reactions, such as PCR or RT-PCR.

Figs. 21A and 21B ~~Figs. 22A and 22B~~ are graphs of steady state power and cooling time versus thermal insulator thickness, respectively, for the device of Fig. 20 ~~Fig. 21~~.

Fig. 22 ~~Fig. 23~~ is a top view of an array of thin-film heaters mounted on a single thermoelectric cooler for independent rapid thermal cycling reactions in the miniature device of Fig. 20 ~~Fig. 21~~.

Fig. 23 ~~Fig. 24~~ is a cross-section view of a hybridization cartridge.

Fig. 24 ~~Fig. 25~~ is a schematic illustration of a sealed pneumatic cartridge having a deformable diaphragm for drawing fluid into or ejecting fluid from a chamber.

Fig. 25 ~~Fig. 26~~ schematically illustrates an array of sealed pneumatic chambers on disposable cartridges.

Fig. 26 ~~Fig. 27~~ is a cross-sectional view of an electrically controlled nucleic acid purification chamber.

Fig. 27 ~~Fig. 29~~ is a cross-sectional view of a miniaturized m-RNA purification system.

Fig. 28 ~~Fig. 30~~ is a sectional view of a cell lysis or nucleic acid fragmentization system incorporating acoustic energy.

Fig. 29 ~~Fig. 31~~ is a partial sectional view of a cartridge adapted for low volume hybridization of high density oligonucleotide arrays.

Figs. 30A-30E ~~Figs. 34A-34E~~ illustrate a system and method for linking two fluid plugs.

Figs. 31A and 31B ~~Figs. 35A and 35B~~ illustrate alternative embodiments of the system of Figs. 30A-30E ~~Figs. 34A-34E~~.

Figs. 32A, 32B and 32C ~~Figs. 38A, 38B and 38C~~ illustrate a chamber adapted for measuring or metering a variable amount of fluid.

Figs. 33A-33E ~~Figs. 39A-39E~~ illustrate a method for measuring a fluid amount with the chamber of Figs. 32A and 32B ~~Figs. 38A and 38B~~.

Fig. 34 ~~Fig. 40~~ illustrates a tapered chamber for linking fluid plugs with surface tension.

Figs. 35A and 35B ~~Figs. 41A and 41B~~ illustrate a stalactite chamber for linking fluid plugs with surface tension.

Figs. 36A and 36B ~~Figs. 42A and 42B~~ illustrate a chamber having a shallow region for linking fluid plugs with surface tension.

Fig. 37A ~~Fig. 43A~~ illustrates a previous fluid mixing/linking structure with a vent membrane.

Fig. 37B ~~Fig. 43B~~ illustrate the inventive fluid mixing/linking structure with a tapered channel leading to the vent membrane.

Fig. 38 ~~Fig. 45~~ illustrates the inventive T-shaped linker structure.

Figs. 39A-39C ~~Figs. 46A-46C~~ illustrate a method for combining fluid plugs with the T-shaped linker structure of figure 46.

Fig. 40 ~~Fig. 47~~ illustrates a microfluidic system incorporating a vented common line.

Fig. 41 ~~Fig. 48~~ illustrates a low volume hybridization system having a movable pneumatically-controlled wall.

Fig. 42 ~~Fig. 49~~ illustrates a low volume hybridization system having a movable pneumatically-controlled pivoting wall.

Fig. 43 ~~Fig. 50~~ illustrates a fluid distribution device using a pneumatic stepper.

Fig. 44A ~~Fig. 55A~~ illustrates a sectional view of a flow through thermal treatment device.

Fig. 44B ~~Fig. 55B~~ illustrates a top view of the flow through thermal treatment device of Fig. 44A ~~Fig. 55A~~.

Fig. 44C ~~Fig. 55C~~ shows the time constant for transient heating through a flow-through thermal device.

Fig. 44D ~~Fig. 55D~~ shows the half-gap required in a flow-through thermal device.

Fig. 45A, 45B, and 45C ~~Fig. 56A, 56B, and 56C~~ illustrate sequential steps in the fabrication of a molded parylene microcapillary.

Fig. 46A ~~Fig. 57A~~ illustrates a surface-acoustic wave transducer matrix.

Fig. 46B ~~Fig. 57B~~ illustrates a flexural plate wave matrix device.

Fig. 47A ~~Fig. 58A~~ illustrates a sectional side view of a silicon and glass hydrophobic vent.

Fig. 47B ~~Fig. 58B~~ illustrates a top view of the gas-liquid separator of Fig. 47A ~~Fig. 58A~~.

Fig. 47C ~~Fig. 58C~~ illustrates a sectional view of a hydrophobic vent fabricated from two silicon substrates.

Fig. 48 ~~Fig. 59~~ illustrates a sectional side view of a microfluidic particle suspension valving arrangement having minimal dead volume.

Fig. 49 ~~Fig. 60~~ illustrates a device for direct electronic detection of hybridization locations on an oligonucleotide probe array.

Fig. 50 ~~Fig. 61~~ illustrates the device of Fig. 50 ~~Fig. 61~~, further comprising a laser or light source for modifying particle impedance.

Fig. 51 ~~Fig. 62~~ illustrates a top view of a polycarbonate cartridge for simultaneously performing preparative reactions including PCR, fragmentation, and labeling on four separate samples PCR reactions.

Fig. 52 ~~Fig. 63~~ illustrates a valve plate adapted to cover the polycarbonate cartridge of Fig. 51 ~~Fig. 62~~.

Fig. 53 ~~Fig. 64~~ illustrates a side sectional view of a reaction cartridge sandwiched between the valve plate of Fig. 52 ~~Fig. 63~~ and a temperature control fixture.

Fig. 54 ~~Fig. 65~~ illustrates a pneumatic manifold for positioning on top of the valve plate of Fig. 52 ~~Fig. 63~~.

Fig. 55A ~~Fig. 66A~~ illustrates a velocity profile in a fluid plug moving through a capillary.

Fig. 55B ~~Fig. 66B~~ illustrates paths of fluid re-circulation in a fluid plug moving through a capillary.

On page 54, line 5, please replace the text with the following:

A schematic representation of a reaction chamber employing this system is shown in Fig. 11A ~~12A~~. In brief, the reaction chamber 1202 includes a fluid inlet 1204 which is sealed from a fluid passage 1206 by a valve 1208. Typically, this valve can employ a variety of structures, as described herein, but is preferably a flexible diaphragm type valve which may be displaced pneumatically, magnetically or electrically. In preferred aspects, the valves are controlled pneumatically, e.g., by applying a vacuum to the valve to deflect the diaphragm away from the valve seat, thereby creating an opening into adjoining passages. At the end opposite from the inlet, is an outlet vent 1210, and disposed across this outlet vent is a porous hydrophobic membrane 1212. A number of different commercially available hydrophobic membranes may be used as described herein, including, e.g., Versapore 200 R membranes available from Gelman Sciences. Fluid introduced into the reaction chamber fills the chamber until it contacts the membrane 1212. Closure of the valve then allows performance of reactions within the reaction chamber without influencing or influence from elements outside of the chamber.

On page 55, line 21, please replace the text with the following:

Fig. 4C ~~Fig. 12C~~ shows a schematic illustration of a device employing a fluid flow system which utilizes hydrophobic membrane bound vents for control of fluid flow. As shown,

the device 1250 includes a main channel (or common channel) 1252. The main channel is fluidly connected to a series of separate chambers 1254-1260. Each of these fluid connections with the main channel 1252 is mediated (opened or closed) by the inclusion of a separate valve 1262-1268, respectively, at the intersection of these fluid connections with the main channel. Further, each of the various chambers will typically include a vent port 1270-1276, which vent ports will typically be bounded by a hydrophobic or poorly wetting membrane. The basic design of this system is reflected in the device schematic shown in FIG. 5, as well, in that it employs a central distribution chamber or channel.

On page 56, lines 34, 35 and 36, please replace the text with the following:

Fig, 11D ~~Fig. 12D~~ shows a top view of a portion of an injection molded substrate for carrying out the operations schematically illustrated in Fig. 11C ~~Fig. 12C~~. As shown, this device includes liquid loading chambers 1278a and 1280a which are in fluid communication with the fluid inlets 1278 and 1280 (not shown). These fluid inlets may typically be fabricated into the injection molded portion, e.g., drilled into the loading chamber, or fabricated into an overlaying planar member (not shown). Also included are reaction chambers 1254, degassing chambers 1256 and 1256a, measuring chambers 1258, and storage chambers 1260. Each of these chambers is fluidly connected to main channel 1252.

On page 69, line 25, please replace the text with the following:

Fig. 12 ~~Fig. 13~~ shows a schematic illustration of a device configuration for performing sample preparation reactions, generally, utilizing the fluid direction systems described herein, e.g., employing external pressures, hydrophobic vents and pneumatic valves. In the configuration shown, four domains of the device are each addressed by an array of valves, e.g., a valve array, with its own common channel. The four domains may generally be defined as: (1) reagent storage; (2) reaction; (3) sample preparation; and (4) post processing, which are fluidically interconnected. The sample preparation domain is typically used to extract and purify nucleic

acids from a sample. As shown, included in the sample preparation domain are 5 reagent inlets that are fluidly connected to larger volume storage vessels, e.g., within the base unit. Examples of such reagents for extraction reactions may include, e.g., 4M guanidine isothiocyanate, 1xTBE and 50:50 EtOH:H_2 O. The two reaction chambers may include, e.g., affinity media for purification of nucleic acids such as glass wool, or beads coated with poly-T oligonucleotides.

On page 70, line 25, please replace the text with the following:

Fig. 13 ~~Fig. 14~~ is a schematic illustration of a device configuration for addressing situations where several reactions are to be carried out under the same thermal conditions, e.g., multiple parallel sample analyses, duplicating multiplex PCR by carrying out several PCR reactions with single primer pairs in parallel followed by recombining them, or cycle sequencing with a variety of primer pairs and/or templates.

On page 71, line 11, please replace the text with the following:

Fig. 15 ~~Fig. 16~~ is a schematic illustration of a miniaturized nucleic acid extraction device for use with a genetic analysis system according to the present invention. The genetic analysis system may be useful for point-of-care diagnostics, forensic identification, large-scale clinical testing and other applications. Such a system is capable of accepting a patient sample such as blood, urine, spitum, or a cheek-swab suspension. In the past, the extraction of nucleic acids from these types of samples was typically carried out on a bench scale in a series of laborious steps. Some of the most complex procedures are those used to separate the nucleic acids from the lysed mixture. For example, messenger RNA comprises only a small fraction (-20%) of the total cell RNA. Purification of m-RNA would be of interest for messenger expression monitoring applications.

On page 71, line 35, please replace the text with the following:

Fig. 15 ~~Fig. 16~~ illustrates a structure for overcoming this difficulty. More, generally, however, the apparatus shown in

On page 72, line 1, please replace the text with the following:

Fig. 15 ~~Fig. 16~~ can be used to separate out selected portions of biological samples.

On page 72, line 34, please replace the text with the following:

Fig. 18 ~~Fig. 19~~ is a schematic illustration of a miniaturized biological sample refinement device for use with a genetic analysis system according to the present invention. It would be desirable to extract nucleic acids from a subset of the cells or other particles in the initial sample. One way to do this is to reduce the sample's complexity by sorting the cells before lysis.

On page 73, lines 4 through 10, please replace the text with the following:

As shown in Fig. 18 ~~Fig. 19~~, biological sample refinement device 1900 comprises a base or cartridge 1901 made of a polymeric material such as polycarbonate (e.g. by injection molding), glass, silicon, etc. Base 1901 defines at least one chamber 1902 with one or more channels 1904. At least one wall 1906 of the chamber 1902 is textured to increase its surface area. In the example shown in Fig. 18 ~~Fig. 19~~, the wall 1906 includes a number of protrusions 1908 extending therefrom that form a number of recessed areas 1910 that increase the surface area of wall 1906. However, it will be understood that a variety of configurations are possible. For example, the wall 1906 may have a plurality of beads or particles (not shown), e.g., CTG, cellulose, or zeolite, adhesively attached thereto.

On page 73, line 26 through page 74, line 25, please replace the text with the following:

In use, a sample such as whole blood is introduced into the chamber 1902 through an inlet channel 1920 under conditions so that the antibodies 1912 bind to the corresponding cell receptors within the sample. The chamber is washed while the cells remain attached, and then the cells are lysed by the introduction of a lysing agent, such as chaotropic salt. Alternatively, the cells may be lysed by heating them in a hypotonic solution, or adding an enzymatic lysing agent such as protenese K. The lysed cells are then drawn from chamber 1902 through inlet channel 1920 or a second outlet channel 1922. Extraction of the total nucleic acid from this lysate is carried out in a subsequent chamber, as discussed above in reference to Fig. 15 ~~Fig. 16~~. Alternatively, the nucleic acid extraction and subsequent amplification (i.e., PCR) may be performed in-situ within chamber 1902. Temperatures for affinity, washing, and lysis are controlled using a heating element (not shown) pressed against one wall of the cartridge 1901.

In another embodiment shown in Fig 26 ~~Fig. 27~~, nucleic acids are moved selectively in an applied electric field owing to their strong negative charge. These moving nucleic acids are captured on a barrier, e.g. a nanoporous material or dialysis membrane, by directing the field through this material. After capture, the cell debris and other undesirable material can be washed away. This process can be repeated to enhance purification.

As shown in Fig.. 26 ~~Fig. 27~~, a nucleotide separation system 2700 includes a base 2702 defining a purification chamber 2704 with an inlet 2706, outlet 2708 and a plurality of "field" channels 2710. System 2700 further includes a barrier 2712 (e.g. a dialysis membrane), which blocks each of the field channels 2710 to create at least two electrolysis chambers 2714, 2715. Positive and negative platinum wire electrodes 2716, 2717 provided in electrolysis chambers 2714, 2715, respectively. Electrodes 2716, 2717 are each coupled to a voltage source 2720 for applying a potential between the electrolysis chambers.

On page 75, line 21, please replace the text with the following:

Referring to Fig. 27 ~~Fig. 29~~ a messenger RNA purification system 2900 includes a sheet 2902, such as polycarbonate, glass, silicon, or polypropelene, polystyrene, polyethylene, acrylic, and commercial polymers, and a substrate 2904 (e.g., silicon)

having a plurality of ridges 2906 between the sheet 2902 and substrate 2904. Preferably, sheet 2902 is a polymer and substrate 2904 is silicon, but such composition is not limiting as other workable compositions are equally possible. The ridges 2906 are preferably formed using reactive ion etching or other conventional techniques. Poly T oligos or other affinity treatment 2912 are attached to ridges 2906, as discussed below. A piezoelectric crystal 2908 is preferably mounted to the polymeric sheet 2902 opposite substrate 2904.

On page 75 line 34 through page 76, line 36, please replace the text with the following:

In use with the present invention, the polymeric sheet 2902 forms a reaction chamber 2910 between its lower surface and ridges 2906 of the silicon substrate. Poly-T oligonucleotides 2912 are tethered to the silicon surface by oxidation, silation and standard DMT chemistry. The piezoelectric crystal 2908 is used to enhance hybridization through acoustic streaming. A filtered nucleic acid containing solution is mixed with salt (e.g., 6.times.SSPE) to increase the ionic strength for hybridization. The salted sample is introduced into chamber 2910. After sufficient time has elapsed for hybridization, the chamber is washed with a clean salt solution, preferably at an elevated temperature. The m-RNA is removed using a weak buffer (or DI water). More, generally, however, the apparatus shown in Fig. 27 ~~Fig. 29~~ can be used to separate out selected portions of biological samples.

In an alternative embodiment, the oligonucleotides may be synthesized directly using either DMT or light activated phosphoramidites, or pre-synthesized oligonucleotides tethered to the surface using streptavidin/avidin coupling or thiol binding to gold. Although the high surface area is preferably formed by ridges 2906, it will be recognized that this high surface area may be formed by a variety of techniques, for example, the high surface area zone may comprise porous silicon, zeolite, RIE etched pillars, silica xerogel, etched glass, sintered particles, glass spheres or other particles.

Another embodiment for controlling the degree of lysis to select DNA and RNA from plasma, cytoplasma, or nucleus will now be described. In this embodiment, (shown

in Fig. 28 ~~Fig. 30~~), a focused acoustic source, such as a piezoelectric crystal 3002 (preferably a lead-zirconium-titanate or lithium niobate piezoelectric ceramic in a focused shape) is coupled to a thinned wall 3020 of a polymeric base 3004 via a fluid filled balloon 3008. An injected molded chamber 3006 within base 3004 includes a plurality of grooves 3010 on a lower surface for enhancing the lysing effect. Alternatively, the channel wall may be shaped with pits, spikes or other structures and textures such as can be made on a glass, silicon, polycarbonate, polypropelene, polystyrene, polyethylene, acrylic, or commercial polymer such as Kapton, Valox, Teflon, ABS, Delrin and the like structure.

On page 80, line 28, please replace the text with the following:

Referring now to Figs. 30-37 ~~Figs. 34-37~~, systems and methods for combining separate fluids in a miniature biological reactor will now be described. Current methods for mending two or more separate fluids typically involve the use of a long channel which contains venting membranes that pass gas and not fluid. By passing two separate fluids through this channel (with a vent between them), gas separating the two fluids can be expelled, thereby combining the fluids. Problems arise with this method when venting membranes get plugged with fluid and stop functioning.

On page 81, lines 1 through 18, please replace the text with the following:

Referring to Figs. 30A-30E ~~Figs. 34A-34E~~, one embodiment of the present invention for combining two separate fluids will now be described. As shown in Fig. 30A ~~Fig. 34A~~, a vacuum is applied to pull up a flexible valve membrane 3400 and to pull a fluid plug A through a fluid passage 3402 to the edges of an opening 3404 below the membrane 3400. As shown in Fig. 30B ~~Fig. 34B~~, air continuously passes through a vent 3406 in the chamber leaving the fluid plug A behind. As shown in Fig. 30C ~~Fig. 34C~~, fluid B is then pushed through fluid passage 3402 where it combines with fluid A on the edges of opening 2404 (Fig. 30D) ~~(Fig. 34D)~~. Pressure

upon flexible valve member 3400 then causes the valve member to reduce the chamber volume to zero (Fig. 30E) ~~(Fig. 34E)~~. The combined fluids A and B are then expelled back downward through fluid passage 3402.

Referring now to Fig. 34 ~~Fig. 40~~, a fluid-gas separation system 4000 includes a chamber 4002 having a generally tear drop shape that tapers at one end. As shown, a pair of passages 4006, 4008 are coupled to an expanded end 4010 of chamber 4002 and a single passage 4012 is coupled to a tapered end 4014 opposite expanded end 4010. Of course, other geometries can be envisioned in this embodiment of the invention, such as multiple passages on either side of chamber 4002 or only single passages on either side. Liquid surface tension will tend to draw the liquid to the tapered end 4014 of chamber 4002.

On page 82, line 11, please replace the text with the following:

Referring now to Figs. 35A and 35B ~~Figs. 41A and 41B~~, a stalactite chamber 4100 is provided according to the present invention for separating gases and liquids and/or for linking separate fluids. As shown, chamber 4100 includes at least one protrusion 4102 that creates a narrow region 4004 within the chamber. Liquids are introduced into chamber 4000 through one or more passages 4006 and drawn into region 4004 by surface tension. The gases pass through region 4004 into one or more outlet passages 4008. The chamber may include any number or arrangement of protrusions or other geometries that create a narrow region therein.

On page 83, line 14 through page 85, line 8, please replace the text with the following:

Fig. 37A ~~Fig. 43A~~ illustrates a previous linker-vent structure 4300 having channel 4302 with a vent conduit 4304. A vent membrane 4306 covers vent conduit 4304. Applicant has found that the amount of liquid trapped in vent conduit 4304 decreases with the conduit length. According to the present invention, the conduit length can be minimized by forming a tapered vent structure 4320 that includes a tapered vent conduit 4322, as shown in Fig. 37B ~~Fig. 43B~~. Tapered vent conduit 4322 can be formed, for example, with a ball-end mill. Alternatively, the

vent conduit may also be completely eliminated by mounting the vent material inside the channel or chamber.

Another embodiment of the present invention is shown in Fig. 38 ~~Fig. 45~~. Liquids with surfactants can change the wetting properties of a vent so that a liquid film adheres to and blocks the vent. Experiments have revealed that blowing gas through the back of the vent will redistribute this liquid film and clear the vent. However, a cleared wetted vent will generally revert back to a choked wetted vent when it comes in contact with bulk liquid.

Referring again to Fig. 38 ~~Fig. 45~~, an alternative linker structure 4500 takes advantage of the above described vent behavior. As shown, linker structure 4500 comprises a vent 4502 coupled to first and second valves 4504, 4506 so as to form a T-shaped linker structure. This T-shaped linker structure can be used to link two fluid plugs. For example, a first fluid plug 4508 is introduced through valve 4504 to vent 4502, as shown in Fig. 39A ~~Fig. 46A~~. The vent is then cleared by blowing air therethrough, which expels an excess part of fluid 4508 through the second valve 4506 (Fig. 39B) ~~(Fig. 46B)~~. A second fluid plug 4510 is then introduced through the second valve 4506 to vent 4502 to link the first and second fluid plugs 4508, 4510 (Fig. 39C) ~~(Fig. 46C)~~. This process has been demonstrated hundreds of times without failures using mock PCR mixes, real reagents, and solutions with up to five times the amount of Tween-20.

In other embodiments, a pair of crossed channels may be used for linking (i.e., no vents). Alternatively, the vent conduit may be minimized by fabricating a thin wall, e.g., from a thin sheet of plastic, such as polycarbonate or polypropylene, bonded to the cartridge, or by adhesive tape bonding the wall to the cartridge and mounting the vent there.

7. Device and Methods for Metering Fluids

Referring to Figs. 32 and 33 ~~Figs. 38 and 39~~, a system and method for measuring and distributing microliter volumes of fluid in biological cartridge systems will now be described. This newly proposed design generates variable microliter sized fluid plugs. As shown in Figs. 32A and 32B ~~Figs. 38A and 38B~~, a small microliter chamber 3800 is machined out of a suitable material, such as plastic. The chamber 3800 has a ballast end 3802 and an open end 3804 with a valve coupled to a common channel 3806.

In use, a fluid plug 3810 is pushed from the common channel 3806 into the closed chamber 3800 using applied air pressure (see Figs. 33A-B) ~~(see Figs. 39A-B)~~. Pressure builds as the plug 3810 moves into the chamber 3800 and the trapped gas in the closed portion is compressed by the incoming fluid (Figs. 33B and 33C) ~~(Figs. 39B and 39C)~~. The fluid will stop when the compressed gas is equal in pressure to the applied pressure (see Fig. 33D) ~~(see Fig. 39D)~~. The valve 3804 is closed and the common line 3806 is then blown out. By defining the relationships between input pressure chamber volume and resulting plug volume, increasing the input pressure will increase the plug volume and vice versa. By increasing or decreasing the pressure, one can vary the dose size. Opening the valve 3804 causes the plug 3810 to be expelled with the same pressure as the original input pressure (Fig. 33E) ~~(Fig. 39E)~~. Alternatively, as is shown in Fig. 32C ~~Fig. 38C~~, a valve 3803 can be provided at the end of chamber 3800 to permit purging of chamber 3800.

On page 85, line 10, please replace the following:

8. Microdevice for Manipulating Polynucleotides

Fig. 19 ~~Fig. 20~~ schematically illustrates a microdevice 2000 for separating out selected portions of biological samples. Microdevice 2000 may be useful in a variety of applications, but is particularly useful for removing the complex genetic background in a sample, ensuring a constant concentration of DNA or RNA using molar dosing or skewing a sequence population of the mixture by melting point to improve analysis by hybridization array by reducing detection dynamic range requirements. This system can also be used for m-RNA extraction or purification.

On page 86, line 6, please replace the text with the following:

As shown in Fig. 19 ~~Fig. 20~~, the support surface of the affinity chamber can be provided by a compressed plug 2200 of glass wool positioned between channels 2004 and 2006 in a fluidic cartridge 2000 such that fluid passing from channel 2004 to 2006, or vice versa, must pass through the plug 2002. Preferably, plug 2002 is positioned in a vertical portion of the channel as

shown. In the alternative embodiment where solid support 2000 comprises a binding surface disposed in an affinity chamber, fluid is passed over this binding surface when moving through the affinity chamber.

On page 88, line 26, please replace the text with the following:

Fig. 20 ~~Fig. 21~~ is a schematic illustration of one embodiment of this principle. As shown, a cartridge 2100 includes a reaction chamber 2106 having at least one relatively thin wall 2108 on at least one side of chamber 2106. The thicknesses of the reaction chamber and walls are minimized to provide reduced thermal mass. The temperature in reaction chamber 2106 is controlled with a thin heater 2109 pressed against the thin wall 2108 of the reaction chamber 2106. The heater 2109 may comprise an inconel or NiCr alloy, carbon, platinum, nickel or their alloys. The heater 2109 may also include a temperature sensor (not shown) such as an RTD made of platinum or nickel, a 2thermocouple, or a heating element that functions similarly to an RTD.

On page 90, line 30, through page 91, line 26, please replace the text with the following:

Referring now to Fig. 22 ~~Fig. 23~~, a heater array layout 2300 for use with a miniaturized genetic analysis system will now be described. As shown, an array of separately addressable, thin-film inconel heaters 2302 are encapsulated in kapton film. These heaters are commercially available from TransLogic of Huntington Beach, Calif. The heater array 2302 is mounted on a single thermoelectric cooler 2306 with a thermal insulator (not shown) on top (e.g., polycarbonate film 0.5 mm thick), as shown in Fig. 20 ~~Fig. 21~~. The reaction chamber height is relatively small (e.g., about 0.0.1 to 1.0 mm) and the reaction chamber upper and lower walls are relatively thin, (e.g., about 0.1 mm).

In use with the present invention, each heater within the array 2302 is used to control the temperature of an individual reaction chamber within the genetic analysis system. The thermoelectric cooler 2306 functions to provide rapid cooling to all of the

reaction chambers. During a rapid thermal reaction, such as PCR, the cooler 2306 is ON throughout the entire reaction. The heater is turned ON to maintain the reaction temperature. When the reaction temperature should be lowered, the heater is turned OFF, and the cooler 2306 rapidly decreases the temperature within the chamber.

10. Hermetically Sealed Microfluidic System

Fig. 24 and 25 ~~Fig. 25 and 26~~ are schematic illustrations of hermetically sealed microfluidic systems for genetic analysis according to the present invention. In general, PCR reactions are extremely sensitive, but produce a high concentration of DNA product. This combination creates the danger of cross-contamination leading to erroneous results. A disposable cartridge may, for example, contaminate an instrument through PCR-product aerosols that could find their way into cartridges used in subsequent tests.

On page 92, lines 1 through 27, please replace the text with the following:

Two approaches appropriate for disposable cartridges are described herein. In the first approach shown in Fig. 24 ~~Fig. 25~~, a disposable cartridge 2500 defines a reaction chamber 2502 with first and second pneumatic ports 2504, and 2506. A hydrophobic vent 2509 extends between one of the ports 2506 and reaction chamber 2502. A deformable diaphragm seal 2510 such as latex or polyimide, covers the porous hydrophobic vent 2509. Fluids can be drawn into, or ejected from, the chamber by applying vacuums or pressures to the pneumatic ports 2504, 2506. Because deformation of the diaphragm seal 2510 is limited, then it must be positioned in the desired orientation before liquid enters the reaction chamber 2502. For example, diaphragm seal 2510 can be positioned in a "fully exhausted" state by pressurizing pneumatic port 2506 and opening diaphragm valve 2511 to eject gas into an empty chamber. This approach can be extended to a linking/mixing chamber structures (described herein).

In a second approach shown in Fig. 25 ~~Fig. 26~~, a disposable cartridge 2600 comprises both fluidic and pneumatic channels. Single vents 2602 or sets of vents are linked to a pneumatic driving chamber that is addressed by a disposable pneumatic manifold 2606. As with the first

approach, a driving chamber membrane 2608 must be appropriately positioned by exhausting gas into other chambers (e.g., a corresponding driving chamber connected to a second chamber cluster). The driving membrane 2608 is addressed by a non-disposable pneumatic port (not shown).

On page 93, line 11, please replace the text with the following:

As is seen in Fig. 29 ~~Fig. 31~~ a hybridization system 3100 includes a base 3102 that defines a hybridization chamber 3122 with a pneumatic port 3110 and a fluidic port 3111. The probe array 3112 is mounted to base 3102 and a thermal control block 3124 for controlling the temperature of probe array 3112 during hybridization. According to the present invention, a composite porous membrane 3120 is positioned a relatively small distance (e.g., 10 to 100 um) from probe array 3112 to create a smaller chamber 3122 therebetween. The porous membrane 3120 preferably comprises a sandwich of hydrophobic material, such as Versapore 200 from Gelman associates, and a thin membrane with neutral wetting properties, such as particle-track etched polycarbonate from Poretics.

On page 94, lines 16 through page 95, line 28 please replace the text with the following:

Fig. 41 ~~Fig. 48~~ illustrates an embodiment of a low volume hybridization system 4800 which avoids the above limitations. Specifically, hybridization system 4800 includes a hybridization chamber 4802 and pneumatic ports 4804 and 4806. A probe array 4812 is mounted to base 4803. A flexible diaphragm 4820 is included and is addressed by pneumatic ports 4804 and 4806 such that movement of flexible diaphragm 4820 operates to decrease the height of hybridization chamber 4802 such that the chamber volume can be expanded for draining and filling operations and contracted for hybridization. Draining and filling of chamber 4802 is accomplished by simultaneously applying a pressure or a vacuum to pneumatic ports 4804 and 4806. Mixing in chamber 4802 during the hybridization stage can be accomplished by

alternatively applying pressures or vacuums to pneumatic ports 4804 and 4806, thus causing separate portions 4820A and 4820B of diaphragm 4820 (proximal pneumatic ports 4804 and 4806, respectively) to flex in a manner such that fluid is squeezed back and forth within the hybridization chamber as the chamber height above diaphragm portions 4820A and 4820B is varied.

Fig. 42 ~~Fig. 49~~ illustrates an alternative embodiment of a very low volume hybridization system 4900 which includes a hybridization chamber 4902 and a pneumatic port 4904. Probe array 4912 is mounted to base 4903. A flexible diaphragm 4920 mounted to a rigid plate 4922 is also included. Flexible diaphragm 4920 extends fully across the top and thereby seals pressure chamber 4905. Rigid plate 4922 has a hinged end 4923 and a free end 4925. Accordingly, rigid plate 4922 pivots about hinged end 4923 as a pressure differential is applied to pneumatic port 4904. Specifically, as the pressure in pressure chamber 4905 is decreased, rigid plate 4922 pivots downwardly at its free end 4925. Correspondingly, as the pressure in pressure chamber 4905 is increased, rigid plate 4922 pivots upwardly at its free end 4925. As such, the dimension of hybridization chamber 4902 can easily be varied by tilting rigid plate 4922 by applying a pressure differential at pneumatic port 4904. Due to the effects of surface tension, hybridization fluid 4930 will tend to collect at the narrow end of hybridization chamber 4902, as shown. Therefore, decreasing the volume of hybridization chamber 4902 by tilting rigid plate 4922 upwardly will cause the fluid to spread across the surface of the flexible diaphragm. As a consequence, repetitive application of a pressure differential in chamber 4902 will cause the rigid plate 4922 to tilt upwardly and downwardly will cause mixing in the fluid as it repetitively spreads out and then retracts across the diaphragm surface. In addition, upward tilting of rigid plate 4922 also reduces the volume of the hybridization chamber 4902. Draining and filling can be accomplished by applying a vacuum to pneumatic port 4904.

On page 96, line 13, please replace the text with the following:

As shown in Fig. 23 ~~Fig. 24~~, a polycarbonate base cartridge 2402 includes a hybridization chamber 2408 and at least one (preferably two) additional electrolysis chambers 2410, 2412 on either side of the hybridization chamber 2408. Electrolysis chambers 2410, 2412 have positive

and negative electrodes 2430, 2432, respectively, (preferably platinum screens) mounted therein. Electrodes 2430, 2432 may also comprise metals other than platinum, carbon, graphite or pyrolitic forms of these materials, conductive polymers and the like. Alternatively, the electrolysis chambers 2410, 2412 may be filled with a solid polymer electrolyte. Electrolysis chambers 2410, 2412 are sealed from the hybridization chamber 2408 with barriers or membranes 2420, 2422. (Barriers 2420 and 2422 can comprise dialysis membranes). As in previous embodiments, the oligonucleotide array 2406 is mounted to cartridge 2402 within chamber 2408, and a fluidic port 2416 fluidly couples chamber 2408 with the remainder of the system.

On page 97, line 15, please replace the text with the following:

As shown in Fig. 40 ~~Fig. 47~~, a microfluidic vent structure 4700 includes first and second chambers 4702, 4704 each coupled to first and second vented common assemblies 4706, 4708. First vented common assembly 4706 includes a pair of valves 4710, 4712 coupled to chambers 4702, 4704 and each other along a common line 4720. Second vented common assembly 4708 also includes a pair of valves 4722, 4724 coupled to the other end of chambers 4702, 4704, respectively, along a common line 4726. In addition, a vent 4730 is positioned along line 4726 between valve 4722 and a purge line, and a third valve 4732 is positioned along line 4726 between valve 4724 and a waste line. Of course, it will be recognized that this system can be modified to include a single reaction chamber, or more than two. In addition, a network of common lines and vented common lines may be used with this system.

On page 100, line 15 through page 101, line 15, please replace the text with the following:

In another aspect of the invention, as is shown in the sectional view of Fig. 44A ~~Fig. 55A~~ and the top view of Fig. 44B ~~Fig. 44B~~, a flow through thermal treatment device 5500 provides precise thermal control in a fluid while minimizing processing area, thus

overcoming these above limitations, as follows. A series of small parallel flow-through silicon chambers 5510 are formed within a silicon cartridge 5520, as shown. Chambers 5510 are preferably etched in a silicon surface using photolithography and etching techniques such as reactive ion etching [RIE]. Alternately, device 5500 can be mounted on a polymeric cartridge (not shown). A coverslip 5530 having inlet hole 5532 and outlet hole 5534 is preferably formed from Pyrex and is anodically bonded over chambers 5510. In one embodiment, coverslip 5530 could instead be made of silicon, preferably being fusion bonded to the device. Chambers 5510 are preferably 10 to 200 um tall and 1 to 20 um wide and have a length from inlet 5532 to outlet 5534 of 0.2 to 5 mm. In an alternate embodiment, chambers 5510 could be replaced by a single large silicon channel, on the order of 500 μm wide and 1 to 100 μm deep. Inlet and outlet ports 5532 and 5534 which pass through coverslip 5530, have a preferable diameter of 0.05 to 2 mm. In alternate embodiments, the inlet and outlet ports can instead pass through the sides of device 5500, rather than through its cover plate on the silicon cartridge 5520. The fluid path through chambers 5510 and ports 5532 and 5534 can preferably be coated with silicon or parylene or surface modified with silanes. Heating and temperature sensing elements 5540, which may comprise thin film sputtered metal resistors, [such as aluminum, platinum, NiCr or nickel], semiconductors, or hybrid structures such as conductive polymer or thin film heaters on kapon, suitable both for heating and sensing, or thermoelectric coolers may also be fabricated on the non-bonding side of the silicon, as shown. The assembled thermal treatment device 5500 is then preferably adhesively bonded to a fluidic control system [not shown] with its cover glass side facing downwardly. Alternatively, attachment of device 5500 to the fluidic control structure can be accomplished by wax, silicone, epoxy, melted polymer, eutectic materials and solder.

On page 101, line 28 through page 102, line 4, please replace the text with the following:

Chambers 5510 must be designed so that the sample flowing therethrough reaches thermal equilibrium. Assuming laminar flow, the time constant τ for this transient heating process is given by the equations:

where Dth is the thermal diffusivity, δ is the channel half-distance, C is the heat capacity, ρ is the density, k is the thermal conductivity of the liquid and the transient time for water as shown in Fig. 44C ~~Fig. 55C~~. To ensure equilibrium, the space time t.sub.space must be at least 10 times the time constant T. Fig. 44D ~~Fig. 55D~~ shows the half-gap required in flow-through heating structure for water, where L=1 mm, w=10, 100 and 500 um.

On page 103, lines 11 through 34, please replace the text with the following:

In another embodiment of the present invention, SURF-CAP molded parylene microcapillary 5600 is fabricated by the sequentially performed steps shown in Figs. 45A, 45B, and 45C ~~Figs. 56A, 56B and 56C~~, respectively.

Referring first to Fig. 45A ~~Fig. 56A~~, a mold part 5602 having etched cavities 5603 is formed from silicon, glass, or other materials using microfabrication techniques such as annistrophic etching, wet chemical isotropic etching, plasma etching or reactive-ion etching (RIE). Alternatively, mold part 5602 can be machined from plastic or metal. A release layer 5604, preferably comprising a soap film, silane, wax, photoresist, oil or thin layer of parylene N, is then optionally coated onto mold part 5602, by spinning, dipping or vapor phase coating.

Referring next to Fig. 45B ~~Fig. 56B~~, a first layer of parylene 5606 is then deposited on a substrate 5608 which is preferably comprised of polycarbonate, silicon, glass, polypropylene or acrylic. Next, mold part 5602 is positioned over substrate 5608 and is preferably held thereon using a clamp or other alignment fixture. Alternatively, the weight of the mold part may alone be sufficient to hold mold part 5602 onto substrate 5608.

Referring next to Fig. 45C ~~Fig. 56C~~, a second parylene layer 5610 is then deposited into the mold cavities 5603. Following this, mold part 5602 is carefully removed from substrate 5608. Accordingly, as shown in Fig. 45C ~~Fig. 56C~~, a finished structure having

On page 105, lines 16 through 35, please replace the text with the following:

Fig. 46A ~~Fig. 57A~~ illustrates first embodiment employing a SAW transducer matrix 5700 according to the present invention, having its transducers positioned in a square-grid pattern. Specifically, transducers 5702 are positioned at locations "a". Similarly, transducers 5704 are positioned at locations "b", transducers 5706 are positioned at locations "c" and transducers 5708 are positioned at locations "d". As transducer pairs 5702:5704, 5702:5706, 5702:5708, 5704:5706, 5704:5708, and 5706:5708 are selectively activated, standing waves are created at node locations between a:b, a:c, a:d, b:c, b:d, and c:d, respectively. Similarly, a particle can be stepped in a second direction (perpendicular to the first direction) by sequentially activating transducer pari 5702:5704 and then 5706:5708. The creation of these standing waves induces the particles to collect at these nodes.

Particles can therefore be stepped in a first direction, for example, moving from location a:c by first activating transducer pair 5702:5706 and then by activating transducer pair 5704:5708. Consequently, as can be appreciated, the SAW transducer matrix of Fig. 46A ~~Fig. 57A~~ can be used

On page 106, line 18, please replace the text with the following:

Fig. 46B ~~Fig. 57B~~ shows a FPW transducer arrangement 5701 for collecting, moving and sorting particles, optionally functioning as a FACS cell sorter, according to the present invention as follows. First and second 3-phase transducers 5750 and 5760, respectively, are positioned next to one another as shown and are driven such that an acoustic streaming velocity passes therealong forming a longitudinally-extending node at region 5770. Biological particles entering at end 5703 of transducer arrangement 5701 are induced to gather at region 5770, move along through the device, and then exit at end 5705. The particles can then be detected optically or electrically as they pass through the device along region 5770. Optionally, the particles may instead be deflected based upon sorting criteria as they exit the device at its end 5705.

On page 107, line 5, please replace the text with the following:

As is shown in Figs. 47A and 47B ~~Figs. 58A and 58B~~, a hydrophobic vent hydrophobic vent structure 5802 is provided. Hydrophobic vent structure 5802 can be fabricated from silicon and glass by a two-step etching process as follows. First, a gap 5804 is etched to pass through silicon substrate 5805 and a depression 5806 is etched thereupon. The dimension of gap 5804 is preferably on the order of 0.1 to 10 um, as controlled by the etching process. A Pyrex glass cap 5810 is then attached on top of silicon substrate 5805, preferably using anodic bonding or adhesives such as epoxy, RTV, or cyannoacrylate. Surface 5811 of glass cap 5810 and surface 5816 of depression 5806 of substrate 5805 are then optionally rendered hydrophobic by silation with hexamethldisilazane (HMDS), or other appropriate silane. It is preferred that the exposed ligand on the silane is a polyfluorinated hydrocarbon. Alternatively, the surfaces can be made hydrophobic by plasma based CVD, followed by a chemical treatment or the deposition of a polymer film (e.g., silicone from a solvent or vapor phase paylene deposition). Accordingly, hydrophobic vent structure 5802 permits gas to pass freely through gap 5804, along the gap between depression 5806 and glass cap 5810 and out exit port 5807. In contrast, fluid flow through this passage is prohibited both by the very small dimensions of this passageway, and the hydrophobic coating of surface 5811 of glass cap 5810 and surface 5816 of depression 5806 of substrate 5805.

On page 107, line 30, please replace the text with the following:

In yet another embodiment, as shown in Fig. 47C ~~Fig. 58C~~, a hydrophobic vent is fabricated of two silicon substrates, 5850 and 5860. Vent capillaries 5855 are annistropically cut through substrate 5850. Photolithography and reactive-ion etching (RIE) or chemical etching are then used to define the vent capillaries, preferably etching them to a depth of 2 to 10 um with capillary width of 0.5 to 10 um. Silicon substrate 5860 is then joined to silicon substrate 5850, preferably using silicon fusion bonding or adhesives such as epoxy, RTV, or cyannoacrylate.

On page 108, line 30, through page 109, line 10 please replace the text with the following:

In an additional embodiment of the present invention, as is shown in the sectional view of Fig. 48 ~~Fig. 48~~, a microfluidic particle suspension valving arrangement 5900 having minimal dead volume and diaphragm contact area is provided, thus overcoming the above limitations. In valving arrangement 5900, an emulsion of particles 5902 is suspended in a liquid which is immiscible with water, [for example, magnetic particles being suspended in oil]. Alternatively, the emulsion can be replaced by a large polymer linked to the particles. As is shown in the side sectional view of FIG. 48, the emulsion is positioned to be trapped in a shallow hydrophobic region 5904 which occludes a flow channel 5906. When valving arrangement 5900 is in an "open" position, fluid and gas flow past the occluding emulsion by temporarily displacing the emulsion. By applying a magnetic field by way of magnet 5908, [or alternately by applying an electric field], the viscosity of emulsion 5902 is dramatically increased and occludes gas and fluid passage through flow channel 5906.

On page 110, line 5, please replace the text with the following:

In an alternative embodiment of the present invention, as shown in Figs. 49 and 50 ~~Fig. 60 and 61~~, direct electronic detection of the hybridization is achieved, as follows. A substrate 6000 has an oligonucleotide probe array positioned thereon. [For ease of illustration, a close-up view of the region containing only two of the individual probes in this array, being probes 6010 and 6012, is shown.] A series of active electrodes 6002 and 6004 and common electrodes 6006 and 6008 are positioned proximal probes 6010 and 6012, respectively, as shown. Unknown target sequences 6050 and 6060 are each tethered to metal particles 6055 and 6065, as shown. Alternatively, the target sequences 6050 and 6060 have a biotin label, and after hybridization, applied thereon with metal particles after hybridization with streptavidin ligands. Hybridization on the array are detected by sensing a shift in the dielectric properties at the locations where the target sequences bind with the probes. In a preferred embodiment, the electrodes are used to measure the complex impedance proximal a location where binding takes place, with the tethered

metal particles dramatically changing the impedance between the electrodes. An important advantage of such direct electronic detection of hybridization locations is that it further enables the scanning process to be miniaturized. A further advantage is that the present system can also be adapted to alternately detect antigen-antibody or receptor binding instead of hybridization.

On page 110, line 30 through page 111, line 28, please replace the text with the following:

In a preferred embodiment of this system, particles 6055 and 6065 are gold particles, however, platinum or nickel particles may also be used. The relative permitivity of these particles is extremely high compared to the solution. The complex impedance of a target sequence as measured between a pair of electrodes 6002 and 6006 or 6004 and 6008 will shift in the presence of the metallic particles. Some variation will appear in the location of the hybridized particle relative to the electrodes. This is illustrated in Fig. 49 ~~Fig. 60~~ by the relative position of metal particles 6055 and 6065 tethered to unknown target sequences 6050 and 6060, as shown. Accordingly, a distribution of sensitivities exist, with the conformation of metal particle 6065 and target 6060 expected to give a higher signal than the conformation of metal particle 6055 to target 6050.

In a second embodiment of this system, as shown in Fig. 50 ~~Fig. 61~~, the particle conductivity [and thus the measured impedance] of target sequences 6150 and 6160 is modified using a laser or other light source 6170. In this embodiment, semiconductor particles 6155 and 6165 which have a low doping density are tethered to target molecules 6150 and 6160 using known art such as silation or post hybridization staining of biotinalated target as described above. Particles 6155 and 6165 are illuminated by light source 6170 which produces a modulated light, thereby generating carriers in the silicon resulting in a time dependent impedance as measured between electrodes 6102 and 6106 or 6104 and 6108. Conductivity modulation of the semiconductor particles provides the following advantages: (1) increased sensitivity by locking in on the light modulation frequency, and (2) multicolor detection using semiconductor particles with different band gaps. An additional advantage of assisting with spatial localization of binding detection is

possible, for example, where a line of electrodes run in one direction and a line of light excitation scans perpendicular to these electrode lines by passing a moving slit opening 6175 over the array.

On page 111, lines 31 and 32, through page 112, line 5, please replace the text with the following:

In another aspect of the invention, as shown in Fig. 51 ~~Fig. 62~~, a polycarbonate cartridge 6200 for performing PCR reactions is provided. When operating with associated instrumentation under computer control, the cartridge is adapted to simultaneously perform the following on four different samples: (1) store DNA se/calf alkaline phosphatase (CIAP) reagent mix (at 4.degree. C.), (2) store TdT reagent Mix (4.degree. C.), (3) carry out P450 multiplex PCR, (4) store sample of PCR product, (5) join and mix PCR product with DNA se/CIAP mix, (6) incubate mixture, (7) store sample of reaction product, (8) join and mix reaction product with TdT reagent mix, and (9) incubate mixture.

On page 112, lines 9 through 31, please replace the text with the following:

The polycarbonate cartridge 6200 has plurality of liquid control ports 6215 which are generally disposed around the perimeter of the cartridge as shown. The polycarbonate cartridge 6200 of Fig. 51 ~~Fig. 62~~ is adapted to be covered by a valve plate 6310 which is shown in Fig. 52 ~~Fig. 63~~. Valve plate 6310 has a plurality of pneumatic ports 6315 disposed therein, as shown. Valve plate 6310 is adapted to be positioned over cartridge 6200 such that each of the valve plate's pneumatic ports 6315 overlap and mate with a liquid control port 6215 of cartridge 6200. Pneumatic ports 6315 can be used either as valves or vents interchangeably.

As is shown in the sectional side view of Fig. 53 ~~Fig. 64~~, cartridge 6200 is preferably sandwiched between valve plate 6310 and a temperature control fixture 6400. A pneumatic manifold 6410 is positioned over valve plate 6310 and is adapted to individually control the

pressure in each of the pneumatic ports 6315. In addition, a sealed air plenum 6450 is formed between manifold 6410 and valve plate 6310. Air plenum 6450 provides both thermal isolation and a downwards pressure force which is desirable for maintaining thermal contact and ensuring cartridge sealing. In one embodiment, air plenum 6450 includes a sealed membrane to prevent gas leakage. In another embodiment, the air plenum is disposed within the valve plate 6310.

Fig. 54 ~~Fig. 65~~ shows a top view of the pneumatic manifold 6410 of Fig. 53 ~~Fig. 64~~.

On page 113, lines 6 through 25, please replace the text with the following:

Homogeneous mixing can be critical to the performance of enzymatic and other reactions. Under capillary flow conditions, however, mixing is difficult as turbulent flow is difficult to achieve. Experimentation has revealed that fluid plugs moving through capillaries experience a recirculating flow as shown in Fig. 55A ~~Fig. 66A~~ which illustrates a velocity profile in a fluid plug moving through a capillary, and Fig. 55B ~~Fig. 66B~~ which illustrates the paths of fluid re-circulation in the fluid plug.

The movement of a fluid plug 6600 through a capillary 6610 must have a net uniform velocity at its leading edge 6602 and also at its trailing edge 6604. As is shown in the velocity profile of Fig. 55A ~~Fig. 66A~~, a parabolic profile is approached across the fluid plug away from the leading and trailing edges, with the fluid moving fastest along centerline 6603 of the fluid plug, and progressively slowing as the side edges of the fluid plug are approached. Observation has revealed that the fluid flows radially outward at its leading edge 6602 and radially inward at its trailing edge 6604, as illustrated in Fig. 55B ~~Fig. 66B~~ to balance the flow. The

On page 115, line 9 through 26, please replace the text with the following:

In another aspect of the present invention, as shown in the side sectional view of Fig. 16 ~~Fig. 17~~, and the top plan views of Figs. 17A and 17B ~~Figs. 18A and 18B~~, a deformable chamber device 1700 having a pneumatic portion 1701 and a fluid portion 1703 is provided. A plurality of reaction chambers 1702, 1704, 1706 and 1708 are formed in fluid portion 1703, as shown.

Chambers 1702, 1704, 1706 and 1708 are provided with various fluid input/output channels 1801, enabling fluid to enter and exit these chambers. Pneumatic portion 1701 and a fluid portion 1703 are bonded together, with a deformable member 1705, which is preferably fabricated from polypropelene or laytex, being disposed therebetween, acting as a flexible chamber wall which seals the pneumatic chamber. Pneumatic chambers 1722, 1724, 1726 and 1728 are provided in pneumatic portion 1701. These pneumatic chambers 1722, 1724, 1726 and 1728 are positioned directly over each of reaction chambers 1702, 1704, 1706 and 1708, respectively, with deformable member 1705 sealing these chambers.

On page 119, line 36, please replace the text with the following:

Transcription reactions performed in the micro-reactor achieved a 70% yield as compared to conventional methods, e.g., same volume in microfuge tube and water bath or PCT thermal cycler. A comparison of in vitro transcription reaction products using a microchamber versus a larger scale control are shown in Fig. 9B ~~Fig. 10B~~.

On page 120, lines 15 through 21, please replace the text with the following:

Amplification of a target nucleic acid was performed with Perkin-Elmer GeneAmp® PCR kit. The reaction chamber was cycled for 20 seconds at 94.degree. C. (denaturing), 40 seconds at 65.degree. C. (annealing) and 50 seconds at 72.degree. C. (extension). Amplification of approximately 10.sup.9 was shown after 35 cycles. Fig. 9C ~~Fig. 10C~~ shows production of amplified product in the microchamber as compared to a control using a typical PCR thermal cycler.

Example 4 – System Demonstration, Integrated Reactions

A microfabricated polycarbonate device was manufactured having the structure shown in Fig. 14A ~~Fig. 15A~~. The device included three discrete vented chambers. Two of the chambers (top and middle) were thermally isolated from the PCR chamber (bottom) to prevent any

denaturation of the RNA polymerase used in IVT reactions at PCR temperatures. Thermal isolation was accomplished by fabricating the chambers more than 10 mm apart in a thin polycarbonate substrate and controlling the temperatures in each region through the use of thermoelectric temperature controllers, e.g., peltier devices.

On page 121, line 16, please replace the text with the following:

The results of PCR and IVT are shown in Fig. 14B ~~Fig. 15B~~, compared with control experiments, e.g., performed in eppendorf tubes.